

“Observations upon the Acquirement of Secondary Sexual Characters, indicating the Formation of an Internal Secretion by the Testicle.”\* By S. G. SHATTOCK and C. G. SELIGMANN. Communicated by Professor J. R. BRADFORD, F.R.S. Received December 14, 1903,—Read January 28, 1904.

*The Problem Stated.*

The question taken up in the present communication may be concisely stated as follows:—

The most prominent and obvious function of the testicle is the formation of the sperm. Under normal circumstances this is discharged; it constitutes, that is to say, an external secretion.

In spermatogenesis the male attributes culminate. There is, however, another element in maleness, of a different kind, less essential, yet in many cases well pronounced, viz., the acquirement of certain external characters which distinguish the male from the female in many groups of living forms.

That the development of such secondary characters is related to some function of the testicle, appears from the results which follow castration when carried out before the advent of sexual maturity. On what, then, does the production of these characters depend?

It is conceivable that the result may be due to a nervous reflex arising out of the physical function of the sexual mechanism. This view our observations seem to us to disprove.

The genesis of the external male characters must, in our opinion, be transferred from the influence of the nervous system to the realm of chemistry. It depends, with more probability, upon the formation of a second secretion by the testicle, the absorption of which into the circulation induces the metabolic changes that reveal themselves as secondary sexual characters.

The suggestion that such an internal secretion might be elaborated by the “interstitial cells,” which lie in groups between the tubuli, was put forward by one of us (S. G. S.)† in 1897.

The experiments to be recorded were, in fact, primarily designed with the object of eliminating any part that might be played by the tubuli in this connection, and so of determining whether any function could be ascribed to the cells named.

They consisted in ligation of the vasa deferentia in the young of certain forms in which the male exhibits marked secondary characters.

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† ‘British Medical Journal,’ Feb. 20, 1897.

It appeared possible that the epithelium of the testicular tubuli would, under these circumstances, on proliferating, undergo degeneration and atrophy from the pressure due to its own accumulation, whereas the interstitial cells of the stroma might remain intact. This result, however, did not ensue, but others, which we venture to record as bearing on the problem under consideration.

The forms selected were a breed of sheep (Herdwick), the male of which is furnished with long recurved horns, of which the female is quite destitute, and the common fowl.

#### *Observations upon Sheep.*

We owe to the kindness of two friends the opportunity of observing many castrated sheep, as well as a certain number of others on which some form of obliteration of the vas deferens had been practised. Besides the horned (Herdwick) sheep already referred to, we made observations upon the hornless Southdown, in which the results, though less striking, are none the less constant.

The results of occlusion of the vasa deferentia in the Herdwick breed have to be compared with those following castration, and both with the normal standard. Lambs of the same age were selected, and the procedures mentioned were carried out at about the same time.

The occlusion of the vas deferens was effected a short way above the testicle by the application of a silk ligature in two places and division of the duct between. The animals were examined at different periods during their growth, and were killed when fully developed at ages of from 10—14 months.

In those *castrated* either no horns appeared externally, and on preparation the skull exhibited only two low osseous tubercles or horn-cores, or very diminutive horns were produced, and beneath them a slightly more prominent core than in the first case.

In the *ewe* of the Herdwick breed there is no external trace of horn, nor does the prepared skull show any osseous core.

As contrasted with the results of castration, those of *vasotomy* are very striking. The horns attain their full size, and the skull its complete male characters, so that the head in no way differs from that of the normal or intact ram.

The form of the skull is modified by castration, not by double vasotomy, the modification in question being obviously correlated with the absence of horns.

The skull of the castrated sheep, or wether, is less rugged, and the bones thinner, but besides such general differences the plane of the os frontis is continued backwards behind the orbits at a very obtuse angle.

In the intact ram, and equally after vasotomy, the plane of the

frontal behind the orbit lies almost at a right angle with the inter-orbital portion of the bone, the horn-cores arising from the upper or horizontal area.

Although the skull generally is thicker in either case than in the wether, this alone does not account for the difference in external form; the cranial cavity presents a corresponding extension in the frontal region.

In the configuration of its skull, as in the absence of horns, the castrated animal precisely resembles the hornless ewe of the breed.

We have studied the effects of the same procedures upon sheep of a well-known pedigree Southdown herd. The result in such animals is less striking than in the Herdwick, partly because each sex is hornless, and partly because amongst Southdowns individual variations in the form of the head are not uncommon: thus, whilst the head of the wether usually offers a marked contrast to that of the ram, in certain cases the characters of the two so nearly approximate that even an expert may find it difficult to distinguish between them, the ram under such circumstances being commonly called "wether-headed."

In the Southdown there is not (as in the Herdwick) any marked difference produced by castration in the form of the forehead, the angle between the pre- and post-orbital portions of the frontal bone being equally obtuse in the vasotomised sheep and the wether, and this, for the reason that both are equally destitute of horns.

That the occlusion of the vas had been complete in all the cases observed, was proved by a careful dissection of the testicles after the animals were killed.

Seeing that the full development of male characters proceeds in spite of double vasotomy, it becomes interesting to inquire into the condition of the testicles and into the sexual physiology of the animals themselves.

To take the latter first. A Southdown, the subject of double vasotomy when a lamb, and kept apart until full grown from any female, was turned loose with a couple of maiden ewes; he at once copulated, erection and intromission being complete. The two ewes were not again admitted to the flock, but were kept apart, with the result that neither afterwards proved to be in lamb. This animal was killed 18 months after the vasotomy. The testicles had, from the first, grown symmetrically, and had reached the normal size; dissection revealed a complete interruption of each vas close above the gland.

In certain cases one of the testicles underwent a marked diminution, *i.e.*, it not merely failed to grow but rapidly wasted. In the other cases both organs attained the full dimensions. These differences are to be ascribed to differences in the condition of the blood-supply; when the vas is cleanly isolated and divided after ligation without the inclusion of vessels, or without the subsequent occurrence of thrombosis, no atrophy of the gland ensues. When atrophy of one testicle arises,

the other suffices singly to bring about the full development of the male characters.

A careful dissection, carried out in all the cases of vasotomy examined and cited, showed that the vas had been completely occluded; not only was its continuity interrupted, but the noose of the ligature was demonstrated on the end of each segment of the divided duct. The epididymis after occlusion of the vas may become notably larger than normal; this is especially obvious in the lower end or globus minor, and is to be ascribed to its over-distention with the secretion transmitted from the body of the gland.

Microscopic sections of the testis of the normal adult Herdwick sheep and of that from the vasotomised animal of the same age and killed at the same date, show similar histological pictures. The tubuli are filled with epithelial cells, and in nearly every one spermatogenesis is in progress.\*

#### *Experiments upon Fowls.*

Even still more striking are the results of double vasotomy in the cockerel of the common fowl. In the fully grown cock the exposure of the vasa deferentia and their ligation is not particularly difficult, but in the young bird it is otherwise; and the results cited are limited to those cases where careful dissection afterwards proved that this difficult procedure had been successfully carried out, the continuity of the duct being found interrupted, and the noose of the ligature discovered at the site of operation. The method of proceeding was as follows:—The vas is exposed in its course over the kidney by a curved incision carried through the lateral wall of the abdomen; the duct having been ligatured as near to the testicle as possible, is then cut across a short distance below the ligated spot, no ligature being placed on the lower segment. The vas of the other side is afterwards similarly dealt with through a second incision carried through the corresponding side of the abdomen. Owing to the difficulties of this operation, vasotomy was in some cases performed on one side only, the testicle of the other being removed. The anæsthetic used was chloroform, and the material of the ligatures, silk.

The results of double vasotomy, or of one-sided vasotomy combined with one-sided castration, are in all cases alike. When carried out upon the young, immature, bird, or cockerel, the development of the male characters proceeds without any notable interruption, and reaches its full degree.

The birds used in the experiments were so young that it needed an

\* It may be incidentally remarked that whilst in the castrated lamb the prostate fails to grow, in those submitted to vasectomy the gland comes to equal in size that of the intact ram. The same is true of the vesiculæ seminales. If one testicle is removed and the vas of the other ligatured and cut across, the prostate and vesiculæ acquire the full size, and this without asymmetry.

expert to determine their sex : examination, moreover, of the testicles removed from cockerels subjected to the combined castration-vasotomy just referred to, as well as of those removed from birds of the same brood, showed that no spermatogenesis had arisen at the age selected for operation. We may adduce examples in order to give the full grounds for the general statement set forth with regard to these experiments.

*Double Vasotomy.*—Impure “Plymouth Rock,” 7—8 weeks old. Nine months after the operation the head was male in type ; neck-hackles well developed ; tail beginning to assume male characters ; spurs indicated. Twelve months after the date of operation the spurs were stout, though short ; head thoroughly male ; neck- and saddle-hackles moderately well developed ; tail short, male in kind, with sickle feathers.

The bird remained in the same condition, and was killed 12 months after the date of the operation. At the autopsy, the testicles were found to be of full size (about that of a pigeon’s egg), and in their general aspect quite normal. In connection with the right there was a spermatocele about as large as a haricot bean ; this, on being punctured, gave exit to a whitish fluid which microscopically showed numerous spermatozoa, some of them motile. The superior segment of the divided vas, or that in connection with the testicle, was dilated ; the upper end of the lower segment was traceable into scar tissue in which it terminated. On the left side there was no spermatocele in connection with the gland, but the tubuli of the epididymis were abnormally evident. On each side the noose of the silk ligature was found *in situ* on the upper segment of the vas, above the level of the lower border of the testicle.

As a second instance we may recount the following :—

*Double Vasotomy.*—“Plymouth Rock,” about 8 weeks old. In the summer of the year following the operation the head and neck-hackles were typically male, saddle-hackles fairly so ; tail short, carried almost vertically, contained a number of short curved feathers ; spurs short and stout. In the winter of the same year the neck- and saddle-hackles were typically male ; tail short, bushy, feathers curved ; spurs long and sharp. The bird was killed in the spring of the following year. Dissection showed the left testicle to be of full size, 4 cm. in longer diameter ; in connection with the upper end of the epididymis is a retention cyst filled with white secretion, and about 1 cm. in diameter ; the epididymis is, as a whole, enlarged from distension. The upper end of the lower segment of the vas terminates a short distance above the lower border of the testicle. The ligature lies *in situ* on the end of the epididymal segment of the vas, which is separated by a distinct interval from the other.

The right testicle is slightly smaller than the left, the epididymis

distended, and the continuity of the vas interrupted; the ligature lies *in situ* on the lower end of the upper segment of the duct.

As an example showing the results of unilateral vasotomy combined with unilateral castration, we may select the following:—

“Buff Orpington,” about 8 weeks old. Nine months after the operation the bird was thoroughly male, the comb and wattles being well developed, as well as the neck-hackles and sickle-feathers of the tail.

Twelve months after the date of operation the spurs were sharp. On being put with a hen the bird immediately copulated, although it had had no previous opportunity of approaching one. Eighteen months after the operation it was killed. On dissection the right testicle was found to be of full size, about 3·5 cm. in the longer diameter; the epididymis was slightly distended. A scraping from the divided body of the gland revealed the presence of spermatozoa. The lower segment of the vas was found to taper off and end quite distinctly about a quarter of an inch below the level of the testicle. The noose of the ligature was covered with a thin layer of connective tissue, and lay on the posterior surface of the organ. The position of the ligature may be explained by the general growth of the gland; this growth would naturally lead to an extension in all directions, and that in the downward direction would, relatively to the testicle, raise the site of the ligature. On the left side no trace of testicle was found. Microscopic examination of the body of the testis from the case of double vasotomy first cited shows the tubuli to be full of cells, and spermatogenesis in high activity, all the typical histological pictures being present. The same holds true of the right testicle from the case of combined vasotomy and castration last detailed.

These results offer a striking contrast to those following a double castration when carried out upon the immature bird. Double castration was performed through a lateral incision on each side, the testicle being exposed to view, and afterwards carefully disconnected from its attachments with fine forceps, and withdrawn. In ideal experiments the gland was withdrawn entire; in others rupture occurred during the process of detachment, the organ being then removed piecemeal.

*Results of incomplete cauponisation.*—In certain of our experiments it happened that the testicle gave way during its detachment, and that minute fragments were unintentionally left behind. Sometimes such remnants, as told by subsequent dissection, were left in their normal position; at others they were dislocated and transplanted upon the adjacent viscera, or abdominal wall. Under such circumstances the cockerel assumed in different degrees the character of the male.

The actual number of gland remnants left at such imperfect operations, and the position of the grafts resulting from their displacement, varied considerably. Thus, in one case the dissection of the fully

grown bird, which had been castrated when from 6—8 weeks old, showed on the left side a spheroidal mass of testicular substance, 2 cm. in diameter, lying in front of the upper part of the kidney, and into the lower end of which the vas deferens is directly traceable. Hanging in the mid line from a loose “mesorchium” is a spheroidal graft 1·5 cm. in diameter. On the right side there is a bi-lobed mass 2·3 cm. in the chief vertical diameter, with the lower end of which the right vas is directly connected; closely adherent to the front of the upper lobe of this, though slightly movable over it, is a spheroidal mass 0·6 cm. in diameter. A further oval nodule 0·7 cm. in chief diameter is closely adherent to the surface of a coil of the small intestine in the neighbourhood of the liver; a scraping from this graft when cut through in the recent state showed large numbers of spermatozoa. Lastly there is a graft of about the same dimensions intimately adherent to the under surface of the liver itself. The external characters acquired by this bird were fully male throughout.

It may be remarked, in passing, that such grafts do not bear classifying with glandular tumours or adenomata, since they do not grow independently of the general requirements of the body. For the whole sum of a series of such grafts and hyperplastic remnants does not exceed the volume of the two fully developed testicles. In this the remnants behave like those of thyroid tissue left experimentally after partial excision of the thyroid gland; or as do the dormant accessory thyroids after the complete removal of a goitre, when the accessory gland after attaining a certain size ceases to increase further; or the process, again, resembles the reproduction and hyperplasia of hepatic tissue which follows partial excision of the liver, of a fourth or even half its bulk.\*

In the most perfect cases of reproduction, each gland attains its full normal size. A bird was castrated when quite young, 6—8 weeks old. Six months later, the comb and wattles presented a medium degree of development; the spurs were very small. Nine months after the date of operation, the spurs were still small, and the general male characters ill developed. Twelve months after the operation, the spurs were short but stout. Seventeen months after the operation, the comb and wattles were thoroughly male, the neck- and saddle-hackles fully developed, and the spurs long, stout and sharp.

The bird was killed 21 months after the date of the operation. Each testis was found to be of normal form and full size; the epididymis well pronounced, and without retention cysts. Each vas was in every respect normal and filled with white secretion, which microscopically showed countless actively moving spermatozoa. The history, as above given, shows a marked delay in the development of the male characters,

\* Ponfick, ‘Centralblatt f. Med. Wiss.,’ 1894; Von Meister, ‘Centralblatt f. Allg. Path. und Anat. Path.,’ 1891.

and indicates that these developed *pari passu* with the reproduction of the testicles, until they ultimately became fully pronounced.

That a comparatively small volume of testicular tissue will suffice to bring about the development of male characters appears from the following result, in which the bird grew to be fully male with the slight exception that the neck-hackles were somewhat less closely set than is normally the case.

"Buff Orpington," of about 8 weeks, at which time double castration was performed. Eight months after the operation, the comb was well developed and bright in colour; the plumage in general, somewhat pale and sparse; neck-hackles moderately developed; spurs small. Eleven months after the operation, the comb and wattles were well developed; neck-hackles moderate; saddle-hackles fairly male; tail feathers beginning to take the male curve; spurs grown to the normal male extent.

The bird was killed 17 months after the operation, its condition being as last noted. Dissection shows on the left side no trace of testicle in its normal position, but an inch and a half lower down, and three quarters of an inch anterior to this spot, there is an oval graft 2.5 cm. in chief diameter, loosely connected with the lateral wall of the abdomen. Above it, separated by a distance of 1.5 cm. and intimately incorporated with the peritoneum, is a second graft 0.5 cm. in chief diameter; and behind or dorsally to this is a further minute nodule 0.2 cm. in diameter, and likewise inseparably adherent to the peritoneum.

The vas is extremely fine and traceable to the vacant, original, site of the testicle. On the right side in the situation of the testis there are two small flattened nodules, the larger, lower, of which, is 0.8 cm. in chief, vertical diameter. Into the lower end of the inferior the vas, diminished in size and empty of secretion, is directly traceable. A third nodule which lay about 1 cm. anteriorly to these and slightly lower in the abdominal cavity was removed for microscopic purposes: scrapings from its divided surface disclosed the presence of spermatozoa.

Histologically the largest graft (that on the left side of the abdomen) shows closely applied tubuli of full size, every one of which presents the histological pictures typical of active spermatogenesis. The lumen of the tubuli contains free spermatozoa. All the cell nuclei are throughout perfectly stained with nuclear dyes, proving that the tissue is living and not in an obsolete or necrotic condition. The amount of inter-tubular stroma is very small, and supports well formed arterioles and other vessels.

The much greater size of the dislocated graft on the left side of the abdomen, and its high state of activity, suggest that it is the chief element



concerned in the production of the male characters. This graft is strictly ductless, and is, moreover, entirely disconnected from its proper nervous relations.

But much smaller grafts than any of these may be met with in imperfect castration, and in such circumstances the male characters are correspondingly ill-pronounced. One must in fact regard the external character of maleness as a quantity which varies proportionally with the amount of gland-tissue present. As an example of a minimal development of such characters associated with a correspondingly small amount of gland-tissue, we may adduce the following observation:—

A cockerel (impure breed of Plymouth Rock) was castrated when about 6 weeks old. The bird was killed 10 months after the date of the operation, when it exhibited the following characters. The head presented no male development of comb or wattles. As indications of maleness, however, are the full development of the neck-hackles, a certain development of saddle-hackles, the presence of a few straggling badly curved feathers amongst those of the tail, and the growth of short blunt spurs on the legs. It may be noted that the occurrence of spurs in the hens of this breed is not known, except in the case of old birds. The bird took no notice of the hens with which it was habitually kept.

On dissection, no trace of either testicle was discovered at the normal site, and no graft, with the exception of a minute nodule the size of a hempseed, which was adherent to the surface of one of the coils of intestine. Microscopic examination of this minute nodule proved it to consist throughout of testicular tubuli distended with epithelial cells and large numbers of spermatozoa, spermatogenesis being in active progress.

### *Conclusions.*

From the fact that in the young of the Herdwick sheep and fowl, occlusion of the vasa deferentia does not inhibit the full acquirement of secondary male characters, it is clear, in the first place, that the discharge of the sperm is not in any way the factor responsible for the production of the characters referred to.

This conclusion admits of being extended to mean that the production of secondary characters is not due to metabolic changes set up by a nervous reflex arising out of the mere physical function of the sexual mechanism. This is made still more forcible by the results of incomplete caponisation in those cases where the grafts were found in situations far removed from the normal, and altogether disconnected from the nerve supply proper to the testicle in its natural position and connections.

Such grafts, devoid as they are of any channels communicating externally, and consisting as they do, of tubuli only, are virtually

ductless glands, and the metabolic results arising from their function must, as in analogous cases elsewhere, be attributed to the elaboration of an internal secretion and its absorption into the general circulation.

What particular cell elements are concerned in the production of such a secretion cannot as yet be stated. Various possibilities arise which demand the test of further experiment.

The function of spermatogenesis, although not itself the whole or sufficient cause, may be the initial factor of a dual or even a more complex process.

It is quite within the bounds of possibility that certain of the epithelial cells within the tubuli may produce a pro-secretin such as is produced within the intestinal epithelium; that the chemical changes accompanying spermatogenesis in other of the cells of the tubule may lead to the conversion of this pro-secretin into a secretin, much as the acid chyme does in the case of the pro-secretin present in the intestinal cells; and that the secretin so formed may, without being shed into the lumen of the tubule, be transferred to the lymph spaces, and thus eventually reach the general circulation, and incite those metabolic changes in distant parts of the body which disclose themselves as secondary sexual characters. The intimate connection that arises in the process of spermatogenesis between the spermatoblasts and the "sustentacular" cells is a phenomenon not yet explained; this phenomenon possibly coincides with the interaction suggested.

In regard to the interstitial cells of the stroma, they have characters so unmistakeably glandular that some secreting function, probably a sexual one, must be assigned to them, and they may, of course, take a part in the elaboration of such a secretion as that suggested.

But the great variation in the proportion of such cells present in different forms of mammals makes it difficult to formulate any hypothesis to test by way of experiment, and we are not as yet in a position to make any statement in regard to them.

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Preparations illustrating the various observations referred to are now in the museum of the Royal College of Surgeons, London.

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